WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Friday, August 27, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L1	Densham-D$.in.	11
☐	L2	(sequenc$ near polynucleotide) same (enzyme or polymerase)	4766
☐	L3	(sequenc$ near polynucleotide) same (enzyme or helicase or primase)	2687
☐	L4	L2 and enzyme activity	1254
☐	L5	L3 and enzyme activity	879
☐	L6	(L4 or L5) and ((conformation$ change) same enzyme)	31
☐	L7	L6 and (solid support)	23
☐	L8	L6 and ((FRET near Pair) or (energy acceptor and energy donor) or (acceptor and donor))	21
☐	L9	(L6 or L7 or L8) and confocal microscopy	3
☐	L10	(L6 or L7 or L8) and fluorescence imag$	0
☐	L11	(L6 or L7 or L8) and polarization	3
☐	L12	fluorescent imaging	598
☐	L13	(L6 or L7 or L8) and fluorescen$ imag$	2
	DB=USPT,DWPI; PLUR=YES; OP=ADJ		
☐	L14	(sequencing near polynucleotide)	293
☐	L15	target same (enzyme or polymerase or helicase or primase)	21063
☐	L16	L15 and (conformational change near enzyme)	64
☐	L17	L16 and (label)	31
☐	L18	L16 and ((FRET near pair) or (acceptor same donor))	6
☐	L19	L18 and (solid support)	2
☐	L20	L17 and solid support	16
☐	L21	(L17 or L18) and (confocal microscopy)	0
	DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L22	L16 and confocal microscop$	1
☐	L23	L16 and polarization	35
☐	L24	L16 and fluorescen$ imag$	0
☐	L25	(fluorescent or fluorescence) near imaging	2210
☐	L26	L25 and L15	179
☐	L27	L26 and L14	6

☐	L28	(sequence$ near polynucleotide) same (enyme near (polymerase or helicase or primase or holoenzyme))	0
☐	L29	(sequence$ near polynucleotide)	22337
☐	L30	(enyme near (polymerase or helicase or primase or holoenzyme))	1
☐	L31	l29 same (polymerase or helicase or primase or holoenzyme)	3081
☐	L32	L31 same conformational change$	1
☐	L33	L31 and conformational change$	310
☐	L34	(polymerase or helicase or primase or holoenzyme) same (fRET pair or (acceptor and donor))	532
☐	L35	l29 and l34	173
☐	L36	l34 and conformation$ chang$	92
☐	L37	L36 and (immobili$ same solid support)	22
☐	L38	l36 and confocal microscopy	0
☐	L39	L36 and fluorescence imaging	3
☐	L40	l36 and anisotrophy	0
☐	L41	L37 and energy transfer	20
☐	L42	(solid support same (immobil$ or attach$) same (polymerase or helicase) same FRET pair)	0
☐	L43	(solid support same (polymerase or helicase) same FRET pair)	0
☐	L44	((solid support) same (polymerase or helicase) same (FRET pair))	0
☐	L45	(solid support) same (polymerase or helicase)	2016
☐	L46	L45 same (enzyme same (doubl$ label$))	0
☐	L47	L45 and (enzyme same (doubl$ label$))	4
☐	L48	L45 and (enzyme same (FRET pair or (acceptor near donor)))	21
☐	L49	l41 and fluorophore	20
☐	L50	L48 and fluorophore	21
☐	L51	6329178.pn. or 6355421.pn.	4
☐	L52	L51 and (FRET pair or (acceptor and donor))	2
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L53	enzyme same (target or polynucleotide or polymer)	55788
☐	L54	L53 same (fluorophore or fluorescent label or fluorescent moiety or acceptor or donor)	2817
☐	L55	L54 and (polynucleotide not label)	190
☐	L56	L54 and (polynucleotide not label$)	61
☐	L57	l54 and (polymerase or helicase or primase or holoenzyme)	1913
☐	L58	L54 and (polymerase or helicase or primase or holoenzyme)	1913
☐	L59	L56 and (polymerase or helicase or primase or holoenzyme)	24
☐	L60	((polymerase or helicase or primase or holoenzyme) same (immobili$ or attach$ or bound) same (fluorescent or fluorephore or tretramethylrhodamine or cy5))	1619

☐	L61	L60 and (l2 or l3)	152
☐	L62	L61 and (conformation$ chang$)	26
☐	L63	L61 and (solid support)	120
☐	L64	L63 and (FRET pair or (acceptor near donor))	37

END OF SEARCH HISTORY

```
MISSING OPERATOR 'SAME (FRET'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s enzyme and (FRET pair or acceptor and donor)
L6         7446 ENZYME AND (FRET PAIR OR ACCEPTOR AND DONOR)

=> s enzyme (5a) (FRET pair or (acceptor  and donor))
PROXIMITY OPERATION NOT ALLOWED
Certain operators may not be nested in combination with other
operators.  A nested operator is valid only when it occurs at the same
level or above the operator outside the nested phrase as determined by
the following precedence list:

                    1.     Numeric
                    2.     (W), (NOTW), (A), (NOTA)
                    3.     (S), (NOTS)
                    4.     (P), (NOTP)
                    5.     (L), (NOTL)
                    6.     AND, NOT
                    7.     OR

For example, '(MONOCLONAL(W)ANTIBOD?)(L)ANTIGEN?' is valid since (W)
is above (L) on the precedence list.  However,
'((THIN(W)LAYER)(L)PHOSPHOLIPID#)(A)LACTONE#' is not valid since (L)
is below (A) on the precedence list.  The only exception is the 'OR'
operator.  This operator may be used in combination with any other
operator.  For example, '(ATOMIC OR NUCLEAR)(W)REACTOR' is valid.

=> s enzyme (5a) (FRET pair)
L7            0 ENZYME (5A) (FRET PAIR)

=> s enzyme (10a) (FRET pair)
L8            0 ENZYME (10A) (FRET PAIR)

=> sd his
SD IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> d his

     (FILE 'HOME' ENTERED AT 11:31:03 ON 27 AUG 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 11:31:18 ON 27 AUG 2004
L1            19 S (POLYMERASE OR HELICASE OR PRIMASE OR HOLOENZYME) AND (FRET P
L2            17 DUP REM L1 (2 DUPLICATES REMOVED)
L3          1739 S (POLYMERASE OR HELICASE OR PRIMASE OR HOLOENZYME) AND (ACCEPT
L4            18 S L3 AND SOLID SUPPORT
L5            16 DUP REM L4 (2 DUPLICATES REMOVED)
L6          7446 S ENZYME AND (FRET PAIR OR ACCEPTOR AND DONOR)
L7             0 S ENZYME (5A) (FRET PAIR)
L8             0 S ENZYME (10A) (FRET PAIR)

=> s l6 and (conforma? chang?)
L9          200 L6 AND (CONFORMA? CHANG?)

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10           81 DUP REM L9 (119 DUPLICATES REMOVED)

=>  s l10 and sequenc?
```

L11 19 L10 AND SEQUENC?

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12 19 DUP REM L11 (0 DUPLICATES REMOVED)

=> d ibib abs l12 1-19

L12 ANSWER 1 OF 19 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-27838 BIOTECHDS
TITLE: New nucleic acid sensor molecule useful for detecting adenosine diphosphate (ADP) and for identifying biological agents that produce or consume ADP useful for treating diseases in which kinase activity is implicated, e.g. type II diabetes;
DNA sensor and RNA **enzyme** and aptamer for use in disease therapy and gene therapy
AUTHOR: DIENER J L; SRINIVASAN J; HAMAGUCHI N; BLANCHARD J; KURZ J; KURZ M; CLOAD S T; FERGUSON A; EPSTEIN D; WILSON C; STANTON M
PATENT ASSIGNEE: ARCHEMIX CORP
PATENT INFO: WO 2003084471 16 Oct 2003
APPLICATION INFO: WO 2003-US10360 3 Apr 2003
PRIORITY INFO: US 2003-437949 2 Apr 2003; US 2002-369680 3 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-812657 [76]
AN 2003-27838 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid sensor molecule (I) comprising a target modulation domain (TMD) which recognizes adenosine diphosphate (ADP); a linker domain and catalytic domain (CD), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a system for detecting ADP comprising (I) and a device able to detect a signal generated by the binding of (I) to its target; (2) detecting or identifying ADP in a sample by contacting it with (I); (3) a diagnostic system for detecting or identifying ADP comprising (I) and a detector; (4) identifying an agent (X) that produces or consumes ADP in a reaction; (5) identifying an agent (Y) that modulates activity of (X); (6) any nucleic acid sensor molecule (Ia) that is 100, especially 1000, times more specific for ADP than for ATP (adenosine triphosphate); (7) ADP-specific aptamers (Ib); (8) a diagnostic system for detecting or identifying ADP comprising at least one (Ib) fixed to a substrate and a detector; and (9) a composition comprising (I) affixed to a substrate.
BIOTECHNOLOGY - Preferred Materials: CD comprises a unit able to generate an optical signal, either a single unit (where **conformational change** occurs when (I) binds) or a combination of two, where the distance between them is altered when (I) binds, e.g. a **donor**/quencher or **donor**/**acceptor** pair. (I) may include a detectable label, e.g. a radioisotope or fluorophore (particularly fluorescein, Dabcyl or green fluorescent protein); an affinity capture tag and/or at least one modified nucleotide. Particularly CD is an endonucleolytically active ribozyme, e.g. a hammerhead or a self-ligating ribozyme, e.g. a 1-, 2- or 3-piece ligase. (I) may be DNA and/or RNA and is any of 8 specified **sequences**. The specification also includes about 90 **sequences** for (Ib).Preferred System: The system of (1) also includes a light source and a processor for manipulating optical signals. Preferred Composition: (I) or (Ib) are formulated in a buffer that may also include an RNase inhibitor (e.g. vanadyl, tRNA or polyU) and is essentially free of RNase. (I) may be fixed to a substrate (e.g. gold, silicon, glass or nylon), covalently, non-covalently or by hybridization to an immobilized oligonucleotide (ON), especially where many (I), e.g. at least 250, are immobilized in the form of an array. A preferred substrate is a multiwell plate containing a scintillant embedded in its

surface. (Ib) may also be biotinylated and is then immobilized by interaction with a streptavidin-coated surface. Preferred Process: In method (2), any change in the signal from by the optical system is detected as an indication of presence of ADP. The method may be made quantitative, and the sample is e.g. an environmental, biological or organic sample; a biohazard material; drug; toxin; flavor or fragrance, e.g. cells, tissues (or their extracts) body fluids etc. Methods (4) and (5) are essentially the same and are used to detect ATP synthase; ATPase or a kinase, especially mitogen-activated protein kinase (MAPK), its kinase kinase, or kinase kinase kinase; also a Raf kinase. Preparation: (Ib) are identified by the Selex process, using ADP as the target. (I) are identified by screening a heterogeneous population of oligonucleotides that contain a randomized **sequence** with a constant **sequence** at least one end, most preferably a **sequence** consisting of a kinase aptamer; randomized linker and catalytic ribozyme.

ACTIVITY - Antidiabetic; Cytostatic; No biological data given.

MECHANISM OF ACTION - None given.

USE - (I), and related aptamers, are used for (diagnostic) detection and quantification of ADP, also to identify biological agents (X) that produce or consume ADP, e.g. ATPsynthase, ATPase or kinases, and agents that modulate activity of (X) (claimed), potentially useful as therapeutic or lead compounds for treating diseases in which kinase activity is implicated, e.g. Hirschsprung's disease; type II diabetes; mastocytosis; cancer and endocrine disorders.

ADVANTAGE - (I) and related aptamers, have very high selectivity for ADP over ATP, e.g. by a factor of 104.

EXAMPLE - A pool of RNA aptamers was prepared comprising a 5'-constant region of 18 nucleotides (nt); a central randomized region of 40 nt and a 3'-constant region of 19 nt. The pool was subjected to 16 rounds of affinity selection against adenosine diphosphate (ADP) and the final selection of **sequences** was amplified by reverse transcription PCR to generate DNA for cloning and **sequencing**. The most frequently recovered **sequence** was (29) GGACGGATCGCGTGATGATACCAGCGATCGCGAGAAGAAAGTAAGAAACGGCTGGATCTCACACACCTCCCTG A (29). (220 pages)

L12 ANSWER 2 OF 19 MEDLINE on STN

ACCESSION NUMBER: 2003326056 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12854954

TITLE: Comparison of the closed conformation of the beta 1,4-galactosyltransferase-1 (beta 4Gal-T1) in the presence and absence of alpha-lactalbumin (LA).

AUTHOR: Ramakrishnan B; Qasba Pradman K

CORPORATE SOURCE: Structural Glycobiology Section, LECB, CCR, National Cancer Institute-Frederick, National Institutes of health, Building 469, Room 221, Frederick, Maryland 21702, USA.

CONTRACT NUMBER: N01-C0-12400

SOURCE: Journal of biomolecular structure & dynamics, (2003 Aug) 21 (1) 1-8.
Journal code: 8404176. ISSN: 0739-1102.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200404

ENTRY DATE: Entered STN: 20030713
Last Updated on STN: 20040415
Entered Medline: 20040414

AB beta 1,4-Galactosyltransferase (beta 4Gal-T1) transfers galactose from UDP-galactose to N-acetylglucosamine (GlcNAc) in the presence of Mn(2+) ion. However, in the presence of alpha-lactalbumin (LA) it transfers Gal to glucose (Glc) instead to GlcNAc. Upon substrate binding, beta 4Gal-T1 undergoes transition, from an open to a closed conformation. Although

both the **acceptor** and **donor** substrates can induce the necessary **conformational changes**, the **enzyme** has been crystallized only in the closed conformation in the presence of its preferred **donor**, UDP-Gal. The closed conformation induced by the sugar acceptors or the less preferred **donor** substrates has been observed only when complexed with LA. The crystal structure of beta 4Gal-T1 in the presence of UDP-Gal was previously determined at 2.8 A resolution. We report here the same structure at 2.3 A resolution, which provides a better description of this closed conformation. We have also further refined the structures of beta 4Gal-T1.LA complexes containing the sugar **acceptor** and the less preferred sugar nucleotide **donor** substrates and compared the **conformational changes** in the **enzyme** induced by substrates with and without LA. Based on the binding of UDP-sugar molecules, a rational hypothesis is proposed for the **conformational changes** induced by the **donor** substrate.

L12 ANSWER 3 OF 19 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-13298 BIOTECHDS
TITLE: Nucleic acid sensor for detecting target molecule, comprises target molecule activation site and optical signaling unit that changes its optical properties upon allosteric modulation sensor after recognition of target; DNA biosensor useful for diagnosis, drug screening and optimization, monitoring and expression profiling
AUTHOR: STANTON M; EPSTEIN D; HAMAGUCHI N
PATENT ASSIGNEE: ARCHEMIX CORP
PATENT INFO: WO 2002022882 21 Mar 2002
APPLICATION INFO: WO 2000-US28835 13 Sep 2000
PRIORITY INFO: US 2000-232454 13 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-393977 [42]
AN 2002-13298 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid sensor molecule (I) comprising a target molecule activation site comprising a structure that recognizes a target molecule and an optical signaling unit including at least one nucleotide coupled to a signaling moiety that changes its optical properties upon allosteric modulation of (I) following recognition of the target molecule, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a diagnostic profile (II) produced using (I); (2) identifying (M1) a nucleic acid sensor molecule, by providing a population of oligonucleotides, where the population comprises oligonucleotides comprising a first region comprising a random nucleotide **sequence**, contacting the population with a target molecule, and identifying the oligonucleotide in the population that changes conformation upon recognizing the target molecules; (3) an oligonucleotide (III) identified by the above said method; (4) a diagnostic system (IV) for detecting a target molecule, comprising (I), and a detector in optical communication with (I), where the detector detects changes in the optical properties of (I); and (5) a kit for detecting a target molecule, comprising (I) in which the optical signaling unit comprises a first nucleotide coupled to a first signaling moiety (S1) and a second nucleotide coupled to a second signaling moiety (S2), and where the signaling moieties change proximity to each other upon allosteric modulation by the target molecule to the target activation site, reagents for attaching the first and second signaling moieties, and, optionally control target molecules and one or more buffers for analyte detection.

WIDER DISCLOSURE - The following are disclosed: (1) identifying a riboreporters such as allosteric ribozymes, signaling aptamers or aptamer beacons for detection of conformational isoforms of nuclear hormone receptors (NHRs); (2) riboreporters identified by the above method; (3)

direct mechanistic assay for the action of small molecule ligand agonism, antagonism or partial antagonism of members of NHR or G-protein coupled receptor (GPCR) family; (4) selecting riboreporters which recognize the **conformational change** upon GTP binding and/or specifically interact with newly exposed G-protein receptor binding sites upon activation; (5) riboreporters raised against protein kinases; and (6) multiple classes of phosphodiesterase (PDE) riboreporters.

BIOTECHNOLOGY - Preferred Molecule: S1 and S2 comprises a fluorescent label and a fluorescent quencher, and recognition of target molecule by (I) results in an increase in detectable fluorescence of the fluorescent label. S1 and S2 comprise fluorescent resonance energy transfer (FRET) **donor** and **acceptor** groups, and recognition of target molecule by (I) results in a change in distance between the **donor** and **acceptor** groups, thereby changing optical properties of the molecule. (I) is a DNA or RNA, including at least one modified nucleic acid. The target molecule is a secreted, membrane-associated or cytosolic polypeptide, where the membrane is a plasma membrane. The polypeptide comprises the amino acid **sequence** of nuclear hormone receptor (NHR), G-protein coupled receptor (GPCR), ligand binding portion of GPCR or phosphodiesterase (PDE). (II) is correlated with a wild-type state, a pathological condition or genetic alteration. A number of biosensor molecules are provided. Two or more of the biosensor molecules are provided in a solution, or bound to a substrate, such as glass, silicon, nitrocellulose, nylon or plastic. At least two members of the number of biosensor molecules recognizes different target molecules. Preferred Method: In M1, oligonucleotides comprise one or more fixed **sequences** coupled to random **sequence**. The fixed **sequences** include at least a portion of a catalytic site for catalyzing a chemical reaction. The method further involves identifying target molecule independent catalytic oligonucleotides in the population that have catalytic activity in the absence of target molecule, removing the oligonucleotides from the population prior to contacting with the target molecule, and optionally repeating the above said steps. The target molecule dependent catalytic oligonucleotides have catalytic activity upon recognizing the molecule. The fixed **sequence** including a **sequence** that facilitates cloning or **sequence** of the oligonucleotides, is a portion of a non-functional catalytic site. The **sequence** is selected from polymerase chain reaction (PCR) primer site, RNA polymerase, primer activation site and a restriction endonuclease recognition site. The oligonucleotide is provided on a replicatable nucleic acid **sequence** e.g. plasmid. The random **sequence** includes a target activation site. Preferred System: (IV) further comprises a light source in optical communication with (I), and a processor for processing optical signals detected by the detector.

USE - (I) is useful for detecting a target molecule associated with a pathological condition or genetic alteration. (I) is useful for identifying a drug compound, by identifying a nucleic acid biosensor-based molecule profile of target molecules associated with a disease trait in a patient, administering a candidate compound to the patient, and monitoring changes in the profile. Alternately, the method involves identifying a number of pathway target molecules, administering a candidate compound to a patient having a disease trait, and monitoring changes in the structure, level or activity of two or more of the pathway target molecules using (I). The profile of target molecules or the changes in the structure is compared to the profile of a reference healthy or diseased population (claimed). (I) is useful in multiple assays, for the detection of target molecule. (IV) is useful in the detection of target molecules associated with disease and for the development of drug effective against disease. (I) is useful in diagnostic applications and drug optimization.

ADVANTAGE - (I) is highly sensitive with the ability to detect as few as 10 to the power of 2-10 to the power of 3 molecules of a target,

and is highly specific, capable of distinguishing between closely related molecules. The target molecules are detected rapidly because recognition by nucleic sensor molecules on the biosensor leads to immediate signal generation. The biosensors are ideal for use in a clinical laboratory, affording simple, easily-automated chemistry during selection and engineering and easily automated chemistry during the detection process. The same biosensors which are used for the diagnostic assays can be used in the development of new drugs. Signaling does not require that target molecule remain bound to the biosensor. Because, a large number of target molecules can be monitored simultaneously, the method provides a way to assess the effects of compound on multiple drug targets simultaneously allowing identification of the most sensitive drug targets associated with the particular trait.

EXAMPLE - None given in the source material. (144 pages)

L12 ANSWER 4 OF 19 MEDLINE on STN
ACCESSION NUMBER: 2002396349 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12011052
TITLE: Structural basis of ordered binding of **donor** and **acceptor** substrates to the retaining glycosyltransferase, alpha-1,3-galactosyltransferase.
AUTHOR: Boix Ester; Zhang Yingnan; Swaminathan G Jawahar; Brew Keith; Acharya K Ravi
CORPORATE SOURCE: Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom.
CONTRACT NUMBER: GM58773 (NIGMS)
SOURCE: Journal of biological chemistry, (2002 Aug 2) 277 (31) 28310-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1GWV; PDB-1GWW; PDB-1GX0; PDB-1GX4
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020730
Last Updated on STN: 20030105
Entered Medline: 20020916

AB Bovine alpha-1,3-galactosyltransferase (alpha3GT) catalyzes the synthesis of the alpha-galactose (alpha-Gal) epitope, the target of natural human antibodies. It represents a family of enzymes, including the histo blood group A and B transferases, that catalyze retaining glycosyltransfer reactions of unknown mechanism. An initial study of alpha3GT in a crystal form with limited resolution and considerable disorder suggested the possible formation of a beta-galactosyl-**enzyme** covalent intermediate (Gastinel, L. N., Bignon, C., Misra, A. K., Hindsgaul, O., Shaper, J. H., and Joziasse, D. H. (2001) EMBO J. 20, 638-649). Highly ordered structures are described for complexes of alpha3GT with **donor** substrate, UDP-galactose, UDP- glucose, and two **acceptor** substrates, lactose and N-acetyllactosamine, at resolutions up to 1.46 A. Structural and calorimetric binding studies suggest an obligatory ordered binding of **donor** and **acceptor** substrates, linked to a **donor** substrate-induced **conformational change**, and the direct participation of UDP in **acceptor** binding. The monosaccharide-UDP bond is cleaved in the structures containing UDP-galactose and UDP-glucose, producing non-covalent complexes containing buried beta-galactose and alpha-glucose. The location of these monosaccharides and molecular modeling suggest that binding of a distorted conformation of UDP-galactose may be important in the catalytic mechanism of alpha3GT.

L12 ANSWER 5 OF 19 EMBASE
on STN
ACCESSION NUMBER: 2002312494 EMBASE

TITLE: Crystal structure of the human estrogen sulfotransferase-PAPS complex. Evidence for catalytic role of Ser(137) in the sulfuryl transfer reaction.
AUTHOR: Pedersen L.C.; Petrotchenko E.; Shevtsov S.; Negishi M.
CORPORATE SOURCE: M. Negishi, Lab. of Reproductive Toxicology, National Institutes of Health, Research Triangle Park, NC 27709, United States. negishi@niehs.nih.gov
SOURCE: Journal of Biological Chemistry, (17 May 2002) 277/20 (17928-17932).
Refs: 24
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Estrogen sulfotransferase (EST) transfers the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to estrogenic steroids. Here we report the crystal structure of human EST (hEST) in the context of the V269E mutant-PAPS complex, which is the first structure containing the active sulfate **donor** for any sulfotransferase. Superimposing this structure with the crystal structure of hEST in complex with the **donor** product 3'-phosphoadenosine 5'-phosphate (PAP) and the **acceptor** substrate 17β-estradiol, the ternary structure with the PAPS and estradiol molecule, is modeled. These structures have now provided a more complete view of the S(N)2-like in-line displacement reaction catalyzed by sulfotransferases. In the PAPS-bound structure, the side chain nitrogen of the catalytic Lys(47) interacts with the side chain hydroxyl of Ser(137) and not with the bridging oxygen between the 5'-phosphate and sulfate groups of the PAPS molecule as is seen in the PAP-bound structures. This **conformational change** of the side chain nitrogen indicates that the interaction of Lys(47) with Ser(137) may regulate PAPS hydrolysis in the absences of an **acceptor** substrate. Supporting the structural data, the mutations of Ser(137) to cysteine and alanine decrease gradually k(cat) for PAPS hydrolysis and transfer activity. Thus, Ser(137) appears to play an important role in regulating the side chain interaction of Lys(47) with the bridging oxygen between the 5'-phosphate and the sulfate of PAPS.

L12 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:856608 CAPLUS
DOCUMENT NUMBER: 138:350409
TITLE: Identification of residues involved in the substrate specificity of human and murine dCK
AUTHOR(S): Usova, Elena V.; Eriksson, Staffan
CORPORATE SOURCE: The Biomedical Centre, Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, Uppsala, S-751 23, Swed.
SOURCE: Biochemical Pharmacology (2002), 64(11), 1559-1567
CODEN: BCPCA6; ISSN: 0006-2952
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Deoxycytidine kinase (dCK) is a salvage pathway **enzyme** that can phosphorylate both pyrimidine and purine deoxynucleosides, including important antiviral and cytostatic agents. Earlier studies showed that there are differences in kinetic properties between human and murine dCK, which may explain differences in toxic effects of nucleoside analogs. To determine if certain substitutions in amino acid **sequences** between human and mouse dCK give these differences in substrate specificity the 14 mutants and hybrid forms of human dCK were studied. All variants were characterized with dCyd, dAdo and dGuo as phosphate acceptors and ATP and UTP as phosphate **donor**. The relative activities with dCyd, dAdo and dGuo were about 70, 20, 30%, resp., with UTP as compared to ATP for

human dCK and 40, 60, 70% for mouse dCK. Among all tested mutants only the triple combination of substitutions Q179R-T184K-H187N (RKN) had a kinetic behavior very similar to mouse dCK. The kinetic patterns with several important nucleoside analogs, such as AraC, CdA, ddC and AraG have also been studied. Results demonstrated 50-70% low relative capacities of the recombinant mouse and triple mutant RKN to phosphorylate this nucleoside analogs compare with human dCK. A model for dCK was used to try to explain the functional role of these amino acid substitutions. According to this model the triple mutant RKN have altered amino acids in a region necessary for **conformational changes** during catalyzes. This may affects the substrate selectivity both for the nucleosides and the phosphate donors.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002243321 EMBASE

TITLE: Crystal structure of β1,4-galactosyltransferase complex with UDP-Gal reveals an oligosaccharide **acceptor** binding site.

AUTHOR: Ramakrishnan B.; Balaji P.V.; Qasba P.K.

CORPORATE SOURCE: P.K. Qasba, Structural Glycobiology Section, Lab. of Exp./Computational Biol. CCR, NCI, Frederick, MD 21702-1201, United States. qasba@helix.nih.gov

SOURCE: Journal of Molecular Biology, (2002) 318/2 (491-502). Refs: 36
ISSN: 0022-2836 CODEN: JMOBAK

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The crystal structure of the catalytic domain of bovine β1,4-galactosyltransferase (Gal-T1) co-crystallized with UDP-Gal and MnCl(2) has been solved at 2.8 A resolution. The structure not only identifies galactose, the **donor** sugar binding site in Gal-T1, but also reveals an oligosaccharide **acceptor** binding site. The galactose moiety of UDP-Gal is found deep inside the catalytic pocket, interacting with Asp252, Gly292, Gly315, Glu317 and Asp318 residues. Compared to the native crystal structure reported earlier, the present UDP-Gal bound structure exhibits a large **conformational change** in residues 345-365 and a change in the side-chain orientation of Trp314. Thus, the binding of UDP-Gal induces a **conformational change** in Gal-T1, which not only creates the **acceptor** binding pocket for N-acetylglucosamine (GlcNAc) but also establishes the binding site for an extended sugar **acceptor** . The presence of a binding site that accommodates an extended sugar offers an explanation for the observation that an oligosaccharide with GlcNAc at the non-reducing end serves as a better **acceptor** than the monosaccharide, GlcNAc. Modeling studies using oligosaccharide acceptors indicate that a pentasaccharide, such as N-glycans with GlcNAc at their non-reducing ends, fits the site best. A **sequence** comparison of the human Gal-T family members indicates that although the binding site for the GlcNAc residue is highly conserved, the site that binds the extended sugar exhibits large variations. This is an indication that different Gal-T family members prefer different types of glycan acceptors with GlcNAc at their non-reducing ends. .COPYRGT. 2002 Elsevier Science Ltd. All rights reserved.

L12 ANSWER 8 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2002:323209 BIOSIS

DOCUMENT NUMBER: PREV200200323209

TITLE: DNA **sequence** determinants for sigma70 directed

binding of RNA polymerase to promoter: Role of sigma domains flexibility.
AUTHOR(S): Niedziela-Majka, Anita [Reprint author]; Heyduk, Tomasz [Reprint author]
CORPORATE SOURCE: Biochemistry and Molecular Biology, School of Medicine, St. Louis University, 1402 S. Grand Blvd., Saint Louis, MO, 63104, USA
SOURCE: FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A159. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology. New Orleans, Louisiana, USA. April 20-24, 2002.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Jun 2002
Last Updated on STN: 5 Jun 2002

AB Affinity of model promoter fragments towards. E. coli RNA polymerase (RNAP) was examined using competition assay employing luminescence resonance energy transfer (LRET) between **donor**-labeled polymerase and **acceptor**-labeled DNA. Model DNA constructs were designed to mimic structures characteristic for different steps of transcription initiation. The -35 and -10 regions were found to be equally important for the initial binding. The relative importance of region -10 increased substantially for model DNA constructs mimicking open complex interactions. LRET between fluorophores attached to specific sigma domains was used to follow sigma **conformation changes** during progression of transcription from initiation to productive elongation. It was observed previously that interaction of sigma with the core **enzyme** induced a major structural re-arrangement of sigma domains. Upon binding to the promoter only a slight change of sigma70 domains arrangement was observed and the same interdomain distances were observed for promoters differing in spacer length. Moreover, relative arrangement of these domains was not changed during transition from open to initiation complex and in complex synthesizing the abortive product. These data suggest that upon formation of the holoenzyme, sigma70 domains become rigidly fixed at positions determined by their interactions with the core **enzyme**.

L12 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:473045 CAPLUS
DOCUMENT NUMBER: 135:73697
TITLE: A bioluminescence resonance energy transfer (BRET) fusion molecule and method of use
INVENTOR(S): Joly, Erik
PATENT ASSIGNEE(S): Biosignal Packard Inc., Can.
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001046694	A2	20010628	WO 2000-CA1513	20001222
WO 2001046694	A3	20011129		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: CA 1999-2292036 A 19991222

AB This invention provides a bioluminescence resonance energy transfer (BRET) fusion mol., and method of use. The fusion mol. comprises three components: a bioluminescent **donor** protein (BDP), a modulator, and a fluorescent **acceptor** mol. (FAM), wherein the FAM can accept energy from the BDP-generated luminescence when these components are in an appropriate spatial relationship and in the presence of an appropriate substrate. The modulator can either influence the proximity/orientation of the BDP and the FAM and thereby the energy transfer between these components, or it can play a different role in affecting the energy transfer between the BDP-generated activated product and the FAM. The fusion protein, Rluc:PKA:EYFP (containing Renilla luciferase fusion protein with a synthetic peptide containing a phosphorylation site for protein kinase A fusion protein with enhanced yellow fluorescent protein), was recombinantly prepared and used in a BRET assay with coelenterazine h derivative (as luminescent substrate). The BRET ratio was forskolin dose-dependent such that the BRET ratio decreased with an increase in the concentration of forskolin.

L12 ANSWER 10 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2000106973 EMBASE

TITLE: Tracking sliding clamp opening and closing during bacteriophage T4 DNA polymerase holoenzyme assembly.

AUTHOR: Alley S.C.; Abel-Santos E.; Benkovic S.J.

CORPORATE SOURCE: S.J. Benkovic, Department of Chemistry, 414 Wartik Laboratory, Pennsylvania State University, University Park, PA 16802, United States. sjb1@psu.edu

SOURCE: Biochemistry, (21 Mar 2000) 39/11 (3076-3090). Refs: 36 ISSN: 0006-2960 CODEN: BICHAW

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The bacteriophage T4 DNA polymerase holoenzyme, consisting of the DNA polymerase (gp43), the sliding clamp (gp45), and the clamp loader (gp44/62), is loaded onto DNA in an ATP-dependent, multistep reaction. The trimeric, ring-shaped gp45 is loaded onto DNA such that the DNA passes through the center of the ring. gp43 binds to this complex, thereby forming a topological link with the DNA and increasing its processivity. Using stopped-flow fluorescence-resonance energy transfer, we have investigated opening and closing of the gp45 ring during the holoenzyme assembly process. Two amino acids that lie on opposite sides of the gp45 subunit interface, W91 and V162C labeled with coumarin, were used as the fluorescence **donor** and **acceptor**, respectively. Free in solution, gp45 has two closed subunit interfaces with W91 to V162-coumarin distances of 19 Å and one open subunit interface with a W91 to V162C-coumarin distance of 40 Å. Making the assumption that the distance across the two closed subunit interfaces is unchanged during the holoenzyme assembly process, we have found that the distance across the open subunit interface is first increased to greater than 45 Å and is then decreased to 30 Å during a 10-step assembly mechanism. The gp45 ring is not completely closed in the holoenzyme complex, consistent with previous evidence suggesting that the C-terminus of gp43 is inserted into the gp45 subunit interface. Unexpectedly, ATP-hydrolysis events are coupled to only a fraction of the total distance change, with **conformational changes** linked to binding DNA and gp43 coupled to the majority of the total distance change. Using the nonhydrolyzable ATP analogue ATP-γ-S results in formation of a

nonproductive gp45. gp44/62 complex; however, adding an excess of ATP to this nonproductive complex results in rapid ATP/ATP-γ-S exchange to yield a productive gp45·gp44/62 complex within seconds.

L12 ANSWER 11 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2000062094 EMBASE
TITLE: Functional properties of the heme propionates in cytochrome c oxidase from Paracoccus denitrificans. Evidence from FTIR difference spectroscopy and site-directed mutagenesis.
AUTHOR: Behr J.; Michel H.; Mantele W.; Hellwig P.
CORPORATE SOURCE: P. Hellwig, Max-Planck-Institut fur Biophysik, Abteilung Molekulare Membranbiologie, Heinrich-Hoffmann-Strasse 7, D-60528 Frankfurt/M., Germany. hellwig@biophysik.uni-frankfurt.de
SOURCE: Biochemistry, (16 Feb 2000) 39/6 (1356-1363).
Refs: 38
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB By specific 13C labeling of the heme propionates, four bands in the reduced-minus-oxidized FTIR difference spectrum of cytochrome c oxidase from Paracoccus denitrificans have been assigned to the heme propionates [Behr, J., Hellwig, P., Mantele, W., and Michel, H. (1998) Biochemistry 37, 7400- 7406]. To attribute these signals to the individual propionates, we have constructed seven cytochrome c oxidase variants using site-directed mutagenesis of subunit I. The mutant enzymes W87Y, W87F, W164F, H403A, Y406F, R473K, and R474K were characterized by measurement of enzymatic turnover, proton pumping activity, and Vis and FTIR spectroscopy. Whereas the mutant enzymes W164F and Y406F were found to be structurally altered, the other cytochrome c oxidase variants were suitable for band assignment in the infrared. Reduced-minus-oxidized FTIR difference spectra of the mutant enzymes were used to identify the ring D propionate of heme a as a likely proton **acceptor** upon reduction of cytochrome c oxidase. The ring D propionate of heme a3 might undergo **conformational changes** or, less likely, act as a proton **donor**.

L12 ANSWER 12 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2000275638 EMBASE
TITLE: **Conformational changes** in activated protein C caused by binding of the first epidermal growth factor-like module of protein S.
AUTHOR: Hackeng T.M.; Yegneswaran S.; Johnson A.E.; Griffin J.H.
CORPORATE SOURCE: T.M. Hackeng, Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037, United States. t.hackeng@bioch.unimaas.nl
SOURCE: Biochemical Journal, (1 Aug 2000) 349/3 (757-764).
Refs: 36
ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The first epidermal growth factor-like module of human plasma protein S (EGF1, residues 76-116) was chemically synthesized and tested for its ability to inhibit the anticoagulant cofactor activity of protein S for

the anticoagulant protease, activated protein C (APC). EGF1 completely inhibited the stimulation of APC activity by protein S in plasma coagulation assays, with 50%, inhibition at approx. 1 μM EGF1, suggesting direct binding of EGF1 to APC. To investigate a direct interaction between EGF1 and APC, fluorescence resonance energy transfer (FRET) experiments were employed. APC labelled in the active site with fluorescein as the **donor**, and phospholipid vesicles containing octadecylrhodamine as the **acceptor**, showed that EGF1 association with APC caused an increase in energy transfer consistent with a relocation of the active site of APC from 94 Å (9.4 nm) to 85 Å above the phospholipid surface (assuming $\kappa 2 = 2/3$). An identical increase in energy transfer between the APC active site-bound fluorescein and phospholipid-bound rhodamine was obtained upon association of protein S or protein S-C4b-binding protein complex with APC. The latter suggests the presence of a ternary complex of protein S-C4b-binding protein with APC on the phospholipid surface. To confirm a direct interaction of EGF1 with APC, rhodamine was covalently attached to the α-N-terminus of EGF1, and binding of the labelled EGF1 to APC was directly demonstrated using FRET. The data suggested a separation between the active site of APC and the N-terminus of EGF1 of 76 Å ($\kappa 2 = 2/3$), placing the APC-bound protein S-EGF1 close to, but above, the phospholipid surface and near the two EGF domains of APC. Thus we provide direct evidence for binding of protein S-EGF1 to APC and show that it induces a **conformational change** in APC.

L12 ANSWER 13 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1999395132 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10464296
TITLE: Structure and function of HNK-1 sulfotransferase. Identification of **donor** and **acceptor** binding sites by site-directed mutagenesis.
AUTHOR: Ong E; Yeh J C; Ding Y; Hindsgaul O; Pedersen L C; Negishi M; Fukuda M
CORPORATE SOURCE: Glycobiology Program, Cancer Research Center, The Burnham Institute, La Jolla, California 92037, USA.
CONTRACT NUMBER: PO1 CA71932 (NCI)
RO1 CA33895 (NCI)
SOURCE: Journal of biological chemistry, (1999 Sep 3) 274 (36) 25608-12.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991007

AB HNK-1 glycan, sulfo-->3GlcAbeta1-->3Galbeta1-->4GlcNAc-->R, is uniquely enriched in neural cells and natural killer cells and is thought to play important roles in cell-cell interaction. HNK-1 glycan synthesis is dependent on HNK-1 sulfotransferase (HNK-1ST), and cDNAs encoding human and rat HNK-1ST have been recently cloned. HNK-1ST belongs to the sulfotransferase gene family, which shares two homologous **sequences** in their catalytic domains. In the present study, we have individually mutated amino acid residues in these conserved **sequences** and determined how such mutations affect the binding to the **donor** substrate, adenosine 3'-phosphate 5'-phosphosulfate, and an **acceptor**. Mutations of Lys(128), Arg(189), Asp(190), Pro(191), and Ser(197) to Ala all abolished the enzymatic activity. When Lys(128) and Asp(190) were conservatively mutated to Arg and Glu, respectively, however, the mutated enzymes still maintained residual activity, and both mutant enzymes still bound to adenosine 3',5'-diphosphate-agarose. K128R and D190E mutant enzymes, on the other

hand, exhibited reduced affinity to the **acceptor** as demonstrated by kinetic studies. These findings, together with those on the crystal structure of estrogen sulfotransferase and heparan sulfate N-deacetylase/sulfotransferase, suggest that Lys(128) may be close to the 3-hydroxyl group of beta-glucuronic acid in a HNK-1 **acceptor**. In contrast, the effect by mutation at Asp(190) may be due to **conformational change** because this amino acid and Pro(191) reside in a transition of the secondary structure of the **enzyme**. These results indicate that conserved amino acid residues in HNK-1ST play roles in maintaining a functional conformation and are directly involved in binding to **donor** and **acceptor** substrates.

L12 ANSWER 14 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1998269104 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9603949
TITLE: Coagulation factor XIIIa undergoes a **conformational change** evoked by glutamine substrate. Studies on kinetics of inhibition and binding of XIIIA by a cross-reacting antifibrinogen antibody.
AUTHOR: Mitkevich O V; Shainoff J R; DiBello P M; Yee V C; Teller D C; Smejkal G B; Bishop P D; Kolotushkina I S; Fickenscher K; Samokhin G P
CORPORATE SOURCE: Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.
CONTRACT NUMBER: HL-16361 (NHLBI)
HL-50355 (NHLBI)
SOURCE: Journal of biological chemistry, (1998 Jun 5) 273 (23) 14387-91.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980723
Last Updated on STN: 19980723
Entered Medline: 19980716

AB Coagulation factor XIIIa, plasma transglutaminase (endo-gamma-glutamine:epsilon-lysine transferase EC 2.3.2.13) catalyzes isopeptide bond formation between glutamine and lysine residues and rapidly cross-links fibrin clots. A monoclonal antibody (5A2) directed to a fibrinogen Aalpha-chain segment 529-539 was previously observed from analysis of end-stage plasma clots to block fibrin alpha-chain cross-linking. This prompted the study of its effect on nonfibrinogen substrates, with the prospect that 5A2 was inhibiting XIIIa directly. It inhibited XIIIa-catalyzed incorporation of the amine **donor** substrate dansylcadaverine into the glutamine **acceptor** dimethylcasein in an uncompetitive manner with respect to dimethylcasein utilization and competitively with respect to dansylcadaverine. Uncompetitive inhibition was also observed with the synthetic glutamine substrate, LGPGQSKVIG. Theoretically, uncompetitive inhibition arises from preferential interaction of the inhibitor with the **enzyme** -substrate complex but is also found to inhibit gamma-chain cross-linking. The conjunction of the uncompetitive and competitive modes of inhibition indicates in theory that this bireactant system involves an ordered reaction in which docking of the glutamine substrate precedes the amine exchange. The presence of substrate enhanced binding of 5A2 to XIIIa, an interaction deemed to occur through a C-terminal segment of the XIIIa A-chain (643-658, GSDMTVTVQFTNPLKE), 55% of which comprises **sequences** occurring in the fibrinogen epitope Aalpha-(529-540) (GSESGIFTNTKE). Removal of the C-terminal domain from XIIIa abolishes the inhibitory effect of 5A2 on activity. Crystallographic studies on recombinant XIIIa place the segment 643-658 in the region of the groove

through which glutamine substrates access the active site and have predicted that for catalysis, a **conformational change** may accompany glutamine-substrate binding. The uncompetitive inhibition and the substrate-dependent binding of 5A2 provide evidence for the **conformational change**.

L12 ANSWER 15 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 97285155 EMBASE
DOCUMENT NUMBER: 1997285155
TITLE: Troponin T and Ca2+ dependence of the distance between Cys48 and Cys133 of troponin I in the ternary troponin complex and reconstituted thin filaments.
AUTHOR: Luo Y.; Wu J.-L.; Gergely J.; Tao T.
CORPORATE SOURCE: Y. Luo, Muscle Research Group, Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA 02114, United States. yinluo@bbri.harvard.edu
SOURCE: Biochemistry, (1997) 36/36 (11027-11035).
Refs: 49
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Contraction of vertebrate striated muscle is regulated by the interaction of Ca2+ with the heterotrimeric protein troponin (Tn), composed of troponin-C (TnC), troponin-I (TnI), and troponin-T (TnT). Although much is known about the Ca2+-induced **conformational changes** in TnC, the Ca2+binding subunit of Tn, little is known about how TnI, the inhibitory subunit, responds to the binding of Ca2+ to TnC. In this work, we used resonance energy transfer to measure the distance between probes attached at Cys48 and Cys133 in the N- and C-terminal domains, respectively, of TnI. A mutant rabbit skeletal TnI, TnI(48/133) (C64S), was constructed by converting Cys64 into Ser. The remaining two thiols at Cys48 and Cys133 were labeled with the fluorescent **donor** 1,5-IAEDANS, and the nonfluorescent **acceptor**, DAB- Mal. We found an interprobe distance of .apprx.41 Å for both uncomplexed TnI and TnI in the binary complex with TnC. This distance increased to 51 Å in the ternary Tn complex with TnT. These distances did not change significantly on binding of Ca2+ to TnC. In the reconstituted thin filament, this distance remained to be 50 Å in the presence of saturating Ca2+, but increased to .apprx.66 Å on removing Ca2+ with EGTA in the presence of Mg2+. Our results indicate firstly that while TnC has only small effects on the global conformation of TnI, the presence of TnT in the ternary Tn complex gives rise to an apparent elongation of TnI. Secondly, whereas there is no detectable Ca2+, dependent change in the global conformation of TnI in the Tn complex free in solution, the removal of Ca2+ caused a substantial separation of the N- and C-terminal TnI regions in the reconstituted thin filament, owing to the interaction between the C-terminal region of TnI and actin in the relaxed state.

L12 ANSWER 16 OF 19 MEDLINE on STN
ACCESSION NUMBER: 97153152 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8999873
TITLE: Examination of substrate binding in thiamin diphosphate-dependent transketolase by protein crystallography and site-directed mutagenesis.
AUTHOR: Nilsson U; Meshalkina L; Lindqvist Y; Schneider G
CORPORATE SOURCE: Department of Medical Biochemistry and Biophysics, Karolinska Institute, Doktorsringen 4, S-171 77 Stockholm, Sweden.
SOURCE: Journal of biological chemistry, (1997 Jan 17) 272 (3)

1864-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1NGS
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970227
Last Updated on STN: 19970227
Entered Medline: 19970213

AB The three-dimensional structure of the quaternary complex of Saccharomyces cerevisiae transketolase, thiamin diphosphate, Ca2+, and the **acceptor** substrate erythrose-4-phosphate has been determined to 2.4 A resolution by protein crystallographic methods. Erythrose-4-phosphate was generated by enzymatic cleavage of fructose-6-phosphate. The overall structure of the **enzyme** in the quaternary complex is very similar to the structure of the holoenzyme; no large **conformational changes** upon substrate binding were found. The substrate binds in a deep cleft between the two subunits. The phosphate group of the substrate interacts with the side chains of the conserved residues Arg359, Arg528, His469, and Ser386 at the entrance of this cleft. The aldehyde moiety of the sugar phosphate is located in the vicinity of the C-2 carbon atom of the thiazolium ring of the cofactor. The aldehyde oxygen forms hydrogen bonds to the side chains of the residues His30 and His263. One of the hydroxyl groups of the sugar phosphate forms a hydrogen bond to the side chain of Asp477. The preference of the **enzyme** for **donor** substrates with D-threo configuration at the C-3 and C-4 positions and for alpha-hydroxylated **acceptor** substrates can be understood from the pattern of hydrogen bonds between **enzyme** and substrate. Amino acid replacements by site-directed mutagenesis of residues Arg359, Arg528, and His469 at the phosphate binding site yield mutant enzymes with considerable residual catalytic activity but increased Km values for the **donor** and in particular **acceptor** substrate, consistent with a role for these residues in phosphate binding. Replacement of Asp477 by alanine results in a mutant **enzyme** impaired in catalytic activity and with increased Km values for **donor** and **acceptor** substrates. These findings suggest a role for this amino acid in substrate binding and catalysis.

L12 ANSWER 17 OF 19 EMBASE
on STN
ACCESSION NUMBER: 95202573 EMBASE
DOCUMENT NUMBER: 1995202573
TITLE: Calcium-induced troponin flexibility revealed by distance distribution measurements between engineered sites.
AUTHOR: Zhao X.; Kobayashi T.; Malak H.; Gryczynski I.; Lakowicz J.; Wade R.; Collins J.H.
CORPORATE SOURCE: Dept. of Biological Chemistry, Univ. of Maryland School of Medicine, 108 N. Greene St.,Baltimore, MD 21201, United States
SOURCE: Journal of Biological Chemistry, (1995) 270/26 (15507-15514).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The contraction of vertebrate striated muscle is regulated by Ca2+ binding to troponin C (TnC). This causes **conformational changes** which alter the interaction of TnC with the inhibitory protein TnI and the tropomyosin- binding protein TnT. We have used the frequency domain method

of fluorescence resonance energy transfer to measure TnT-TnC and TnT-TnI distances and distance distributions, in the presence of Ca2+, Mg2+, or EGTA, in TnC · TnI · TnT complexes. We reconstituted functional, ternary troponin complexes using the following recombinant subunits whose **sequences** were based on those of rabbit skeletal muscle: wild-type TnC; TnT25, a mutant C-terminal 25- kDa fragment of TnT containing a single Trp212 which was used as the sole **donor** for fluorescence energy transfer measurements; Trp-less ThI mutants which contained either no Cys or a single Cys at position 9, 96, or 117. Energy **acceptor** groups were introduced into TnC or TnI by labeling with dansyl aziridine or N-(iodoacetyl)-N'-(1-sulfo-5-naphthyl)ethylenediamine. Our results indicate that the troponin complex is relatively rigid in relaxed muscle, but becomes much more flexible when Ca2+ binds to regulatory sites in TnC. This increased flexibility may be propagated to the whole thin filament, releasing the inhibition of actomyosin ATPase activity and allowing the muscle to contract. This is the first report of distance distribution measurements between troponin subunits.

L12 ANSWER 18 OF 19 MEDLINE on STN
ACCESSION NUMBER: 95255273 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7737165
TITLE: Oligosaccharyl transferase is a constitutive component of an oligomeric protein complex from pig liver endoplasmic reticulum.
AUTHOR: Breuer W; Bause E
CORPORATE SOURCE: Institut fur Physiologische Chemie, Bonn, Germany.
SOURCE: European journal of biochemistry / FEBS, (1995 Mar 15) 228 (3) 689-96.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950615
Last Updated on STN: 19950615
Entered Medline: 19950602

AB Oligosaccharyl transferase (OST), an intrinsic component of the endoplasmic reticulum membrane, catalyses the N-glycosylation of specific asparagine residues in nascent polypeptide chains. We have purified the **enzyme** from crude pig liver microsomes by a procedure involving salt/detergent extraction, concanavalin-A precipitation, S-Sepharose, MonoP and concanavalin-A-Sepharose chromatographies. A highly purified OST preparation exerting catalytic activity, contained two protein subunits of 48 kDa and 66 kDa, from which the 66-kDa species was identified by immunoblotting as ribophorin I. The function of ribophorin I in this dimeric protein complex is unknown. The high degree of similarity between its transmembrane region and a putative dolichol-recognition consensus **sequence** suggests that ribophorin I could be involved in glycolipid binding and delivery. Several lines of evidence indicate that the catalytically active 48-kDa/66-kDa polypeptides are associated in the endoplasmic reticulum membrane with other proteins, including ribophorin II and a 40-kDa glycoprotein. The implication of ribophorins I and II in the translocation machinery and their apparent association with the OST activity point to a close relationship between polypeptide synthesis, translocation and N-glycosylation, both spacially and temporally. Kinetic studies with the MonoP-purified oligosaccharyl transferase showed that the **enzyme** transfers dolichyl-diphosphate-linked GlcNAc2 to synthetic tripeptides and hexapeptides, containing the Asn-Xaa-Thr motif, at a comparable rate. The glycosylation reaction was found to have a pH optimum close to 7 and to require divalent metal ions, with Mn2+ being most effective. Substitution of threonine in the N-glycosylation motif by serine impairs its function as an **acceptor**, measured by Vmax/Km, by approximately 17-fold,

consisting of a 7.3-fold increase in Km and a 2.3-fold decrease in Vmax. This indicates that the side chain structure of the hydroxyamino acid influences both binding and catalysis, consistent with previous studies highlighting its participation in the catalytic mechanism of transglycosylation. The Km values of peptide acceptors improved significantly when dolichyl-phosphate-bound oligosaccharides were used instead of lipid-linked GlcNAc2 as the glycosyl **donor**. We conclude from this observation that the sugar residues on the outer branches of the glycolipid **donor** induce **conformational changes** in the active site of the oligosaccharyl transferase, thus influencing the association constant of the peptide substrate.

L12 ANSWER 19 OF 19 MEDLINE on STN
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AB Site-directed mutagenesis was used to examine the catalytic importance of 2 histidine and 4 arginine residues in Escherichia coli periplasmic acid phosphatase (EcAP). The residues that were selected as targets for mutagenesis were those that were also conserved in a number of high molecular weight acid phosphatases from eukaryotic organisms, including human prostatic and lysosomal acid phosphatases. Both wild type EcAP and mutant proteins were overproduced in E. coli using an expression system based on the T7 RNA polymerase promoter, and the proteins were purified to homogeneity. Examination of the purified mutant proteins by circular dichroism and proton NMR spectroscopy revealed no significant **conformational changes**. The replacement of Arg16 and His17 residues that were localized in a conserved N-terminal RHGXRXP motif resulted in the complete elimination of EcAP enzymatic activity. Critical roles for Arg20, Arg92, and His303 were also established because the corresponding mutant proteins exhibited residual activities that were not higher than 0.4% of that of wild type **enzyme**. In contrast, the replacement of Arg63 did not cause a significant alteration of the kinetic parameters. The results are in agreement with a previously postulated distant relationship between acid phosphatases, phosphoglycerate mutases, and fructose-2,6-bisphosphatase. These and earlier results are also consistent with the conclusion that 2 histidine residues participate in the catalytic mechanism of acid phosphatases, with His17 playing the role of a nucleophilic **acceptor** of the phospho group, whereas His303 may act as a proton **donor** to the alcohol or phenol.

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